

WHAT IS CLAIMED IS:

1. A method for detecting the presence or absence of *B. anthracis* in a biological
 5 sample from an individual or in a non-biological sample, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an
 amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said
 sample with a pair of *capB* primers to produce a *capB* amplification product if a *B. anthracis*
capB nucleic acid molecule is present in said sample, wherein said hybridizing step comprises
 10 contacting said sample with a pair of *capB* probes, wherein the members of said pair of *capB*
 probes hybridize within no more than five nucleotides of each other, wherein a first *capB* probe
 of said pair of *capB* probes is labeled with a donor fluorescent moiety and said second *capB*
 probe of said pair of *capB* probes is labeled with a corresponding acceptor fluorescent moiety;
 and

15 detecting the presence or absence of fluorescence resonance energy transfer
 (FRET) between said donor fluorescent moiety of said first *capB* probe and said acceptor
 fluorescent moiety of said second *capB* probe,

wherein the presence of FRET is indicative of the presence of *B. anthracis* in said
 sample, and wherein the absence of FRET is indicative of the absence of *B. anthracis* in said
 sample.

2. The method of claim 1, wherein said pair of *capB* primers comprises a first *capB*
 primer and a second *capB* primer, wherein said first *capB* primer comprises the sequence

5'-CCC AAT TCG AGT AAA CAT A-3' (SEQ ID NO:1), and wherein said second

25 *capB* primer comprises the sequence

5'-ACT GCC ATA CAT TCA CAA-3' (SEQ ID NO:2).

3. The method of claim 1, wherein said first *capB* probe comprises the sequence

5'-CGA TTA AGC GCC GTA AAG AAG GTC CTA ATA TC-3' (SEQ ID NO:3), and

30 wherein said second *capB* probe comprises the sequence

5'-GTG AGC AAC GCA GGG TAG TTA AAG AGG CTG-3' (SEQ ID NO:4).

4. The method of claim 1, wherein the members of said pair of *capB* probes hybridize within no more than two nucleotides of each other.

5. The method of claim 1, wherein the members of said pair of *capB* probes hybridize within no more than one nucleotide of each other.

6. The method of claim 1, wherein said donor fluorescent moiety is fluorescein.

7. The method of claim 1, wherein said corresponding acceptor fluorescent moiety is selected from the group consisting of LC-Red 640, LC-Red 705, Cy5, and Cy5.5.

8. The method of claim 1, wherein said detecting step comprises exciting said sample at a wavelength absorbed by said donor fluorescent moiety and visualizing and/or measuring the wavelength emitted by said acceptor fluorescent moiety.

9. The method of claim 1, wherein said detecting comprises quantitating said FRET.

10. The method of claim 1, wherein said detecting step is performed after each cycling step.

11. The method of claim 1, wherein said detecting step is performed in real time.

12. The method of claim 1, further comprising determining the melting temperature between one or both of said *capB* probe(s) and said *capB* amplification product, wherein said melting temperature confirms said presence or said absence of said *B. anthracis*.

13. The method of claim 1, wherein the presence of said FRET within 45 cycling steps is indicative of the presence of a *B. anthracis* infection in said individual.

14. The method of claim 1, wherein the presence of said FRET within 40 cycling steps is indicative of the presence of a *B. anthracis* infection in said individual.

15. The method of claim 1, wherein the presence of said FRET within 30 cycling steps is indicative of the presence of a *B. anthracis* infection in said individual.

16. The method of claim 1, further comprising: preventing amplification of a contaminant nucleic acid.

17. The method of claim 16, wherein said preventing comprises performing said amplifying step in the presence of uracil.

18. The method of claim 17, wherein said preventing further comprises treating said sample with uracil-DNA glycosylase prior to a first amplifying step.

19. The method of claim 1, wherein said biological sample is selected from the group consisting of dermal swabs, cerebrospinal fluid, blood, sputum, bronchio-alveolar lavage, bronchial aspirates, and feces.

20. The method of claim 1, wherein said non-biological sample is selected from the group consisting of powders, filtered air samples, surface swipes, and rinse products from solid materials.

21. The method of claim 1, further comprising:
performing at least one cycling step, wherein said cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of *pagA* primers to produce a *pagA* amplification product if a *B. anthracis* *pagA* nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of *pagA* probes, wherein the members of said pair of *pagA* probes hybridize within no more than five nucleotides of each other, wherein a first *pagA* probe of said pair of *pagA* probes is labeled with a donor fluorescent moiety and said second *pagA*

probe of said pair of *pagA* probes is labeled with a corresponding acceptor fluorescent moiety;
and

detecting the presence or absence of FRET between said donor fluorescent moiety
of said first *pagA* probe and said acceptor fluorescent moiety of said second *pagA* probe.

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22. The method of claim 21, wherein said pair of *pagA* primers comprises a first *pagA*
primer and a second *pagA* primer, wherein said first *pagA* primer comprises the sequence

5'-TAC AGG ACG GAT TGA TAA G-3' (SEQ ID NO:5), and wherein said second
pagA primer comprises the sequence

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5'-TTT CAG CCC AAG TTC TTT-3' (SEQ ID NO:6).

23. The method of claim 21, wherein said first *pagA* probe comprises the sequence
5'-AGT ACA TGG AAA TGC AGA AGT G-3' (SEQ ID NO:7), and wherein said
second *pagA* probe comprises the sequence

5'-ATG CGT CGT TCT TTG ATA TTG GT-3' (SEQ ID NO:8).

24. The method of claim 21, further comprising:

performing at least one cycling step, wherein said cycling step comprises an
amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said
sample with a pair of *lef* primers to product a *lef* amplification product if a *B. anthracis lef*
nucleic acid molecule is present in said sample, wherein said hybridizing step comprises
contacting said sample with a pair of *lef* probes, wherein the members of said pair of *lef* probes
hybridize within no more than five nucleotides of each other, wherein a first *lef* probe of said pair
of *lef* probes is labeled with a donor fluorescent moiety and said second *lef* probe of said pair of
lef probes is labeled with a corresponding acceptor fluorescent moiety; and

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detecting the presence or absence of FRET between said donor fluorescent moiety
of said first *lef* probe and said acceptor fluorescent moiety of said second *lef* probe.

25. The method of claim 24, wherein said pair of *lef* primers comprises a first *lef*
primer and a second *lef* primer, wherein said first *lef* primer comprises the sequence

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5'-TTT TAC CGA TAT TAC TCT CC-3' (SEQ ID NO:9), and wherein said second *lef* primer comprises the sequence

5'-AAC CTA AAG GCT TCT GC-3' (SEQ ID NO:10).

26. The method of claim 24, wherein said first *lef* probe comprises the sequence 5'-ATT AAG GAA TGA TAG TGA GGG T-3' (SEQ ID NO:11), and wherein said second *lef* probe comprises the sequence

5'-TAT ACA CGA ATT TGG ACA TGC T-3' (SEQ ID NO:12).

27. The method of claim 1, wherein said cycling step is performed on a control sample.

28. The method of claim 27, wherein said control sample comprises said portion of said *B. anthracis capB* nucleic acid molecule.

29. The method of claim 1, wherein said cycling step uses a pair of control primers and a pair of control probes, wherein said control primers and said control probes are other than said *capB* primers and *capB* probes, wherein said amplifying step produces a control amplification product, wherein said control probes hybridize to said control amplification product.

30. A method for detecting the presence or absence of *B. anthracis* in a biological sample from an individual or in a non-biological sample, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of *pagA* primers to produce a *pagA* amplification product if a *B. anthracis pagA* nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of *pagA* probes, wherein the members of said pair of *pagA* probes hybridize within no more than five nucleotides of each other, wherein a first *pagA* probe of said pair of *pagA* probes is labeled with a donor fluorescent moiety and said second *pagA*

probe of said pair of *pagA* probes is labeled with a corresponding acceptor fluorescent moiety;
and

detecting the presence or absence of fluorescence resonance energy transfer
(FRET) between said donor fluorescent moiety of said first *pagA* probe and said acceptor
5 fluorescent moiety of said second *pagA* probe,

wherein the presence of FRET is indicative of the presence of *B. anthracis* in said
sample, and wherein the absence of FRET is indicative of the absence of *B. anthracis* in said
sample.

10 31. A method for detecting the presence or absence of *B. anthracis* in a biological
sample from an individual or in a non-biological sample, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an
amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said
sample with a pair of *lef* primers to produce a *lef* amplification product if a *B. anthracis lef*
15 nucleic acid molecule is present in said sample, wherein said hybridizing step comprises
contacting said sample with a pair of *lef* probes, wherein the members of said pair of *lef* probes
hybridize within no more than five nucleotides of each other, wherein a first *lef* probe of said pair
of *lef* probes is labeled with a donor fluorescent moiety and said second *lef* probe of said pair of
lef probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of fluorescence resonance energy transfer
(FRET) between said donor fluorescent moiety of said first *lef* probe and said acceptor
20 fluorescent moiety of said second *lef* probe,

wherein the presence of FRET is indicative of the presence of *B. anthracis* in said
sample, and wherein the absence of FRET is indicative of the absence of *B. anthracis* in said
25 sample.

32. An article of manufacture, comprising:

a pair of *capB* primers;

a pair of *capB* probes; and

30 a donor fluorescent moiety and a corresponding acceptor fluorescent moiety.

33. The article of manufacture of claim 32, wherein said pair of *capB* primers comprise a first *capB* primer and a second *capB* primer, wherein said first *capB* primer comprises the sequence

5'-CCC AAT TCG AGT AAA CAT A-3' (SEQ ID NO:1), and wherein said second *capB* primer comprises the sequence

5'-ACT GCC ATA CAT TCA CAA-3' (SEQ ID NO:2).

34. The article of manufacture of claim 32, wherein said pair of *capB* probes comprises a first *capB* probe and a second *capB* probe, wherein said first *capB* probe comprises the sequence

5'-CGA TTA AGC GCC GTA AAG AAG GTC CTA ATA TC-3' (SEQ ID NO:3), and wherein said second *capB* probe comprises the sequence

5'-GTG AGC AAC GCA GGG TAG TTA AAG AGG CTG-3' (SEQ ID NO:4).

35. The article of manufacture of claim 34, wherein said first *capB* probe is labeled with said donor fluorescent moiety and wherein said second *capB* probe is labeled with said corresponding acceptor fluorescent moiety.

36. The article of manufacture of claim 32, further comprising a package insert having instructions thereon for using said pair of *capB* primers and said pair of *capB* probes to detect the presence or absence of *B. anthracis* in a sample.

37. The article of manufacture of claim 32, further comprising:
a pair of *pagA* primers;
a pair of *pagA* probes; and
a donor fluorescent moiety and a corresponding acceptor fluorescent moiety.

38. An article of manufacture, comprising:
a pair of *pagA* primers;
a pair of *pagA* probes; and
a donor fluorescent moiety and a corresponding acceptor fluorescent moiety.

39. The article of manufacture of claim 38, wherein said pair of *pagA* primers comprises a first *pagA* primer and a second *pagA* primer, wherein said first *pagA* primer comprises the sequence

5 5'-TAC AGG ACG GAT TGA TAA G-3' (SEQ ID NO:5), and wherein said second *pagA* primer comprises the sequence

5'-TTT CAG CCC AAG TTC TTT-3' (SEQ ID NO:6).

40. The article of manufacture of claim 38, wherein said pair of *pagA* probes comprises a first *pagA* probe and a second *pagA* probe, wherein said first *pagA* probe comprises the sequence

5'-AGT ACA TGG AAA TGC AGA AGT G-3' (SEQ ID NO:7), and wherein said second *pagA* probe comprises the sequence

5'-ATG CGT CGT TCT TTG ATA TTG GT-3' (SEQ ID NO:8).

41. The article of manufacture of claim 40, wherein said first *pagA* probe is labeled with a donor fluorescent moiety and wherein said second *pagA* probe is labeled with an acceptor fluorescent moiety.

42. The article of manufacture of claim 38, further comprising a package insert having instructions thereon for using said pair of *pagA* primers and said pair of *pagA* probes to detect the presence or absence of *B. anthracis* in a sample.

43. An article of manufacture, comprising:

a pair of *lef* primers;

a pair of *lef* probes; and

a donor fluorescent moiety and a corresponding acceptor fluorescent moiety.

44. The article of manufacture of claim 43, wherein said pair of *lef* primers comprises a first *lef* primer and a second *lef* primer, wherein said first *lef* primer comprises the sequence

5'-TTT TAC CGA TAT TAC TCT CC-3' (SEQ ID NO:9), and wherein said second *lef* primer comprises the sequence

5'-AAC CTA AAG GCT TCT GC-3' (SEQ ID NO:10).

5 45. The article of manufacture of claim 43, wherein said pair of *lef* probes comprises a first *lef* probe and a second *lef* probe, wherein said first *lef* probe comprises the sequence

5'-ATT AAG GAA TGA TAG TGA GGG T-3' (SEQ ID NO:11), and wherein said second *lef* probe comprises the sequence

5'-TAT ACA CGA ATT TGG ACA TGC T-3' (SEQ ID NO:12).

10 46. The article of manufacture of claim 45, wherein said first *lef* probe is labeled with a donor fluorescent moiety and wherein said second *lef* probe is labeled with an acceptor fluorescent moiety.

15 47. The article of manufacture of claim 43, further comprising a package insert having instructions thereon for using said pair of *lef* primers and said pair of *lef* probes to detect the presence or absence of *B. anthracis* in a sample.

20 48. A method for detecting the presence or absence of *B. anthracis* in a biological sample from an individual or in a non-biological sample, said method comprising:

25 performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of *capB* primers to produce a *capB* amplification product if a *B. anthracis capB* nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a *capB* probe, wherein the *capB* probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety; and

30 detecting the presence or absence of fluorescence resonance energy transfer (FRET) between said donor fluorescent moiety and said acceptor fluorescent moiety of said *capB* probe,

wherein the presence or absence of fluorescence is indicative of the presence or absence of *B. anthracis* in said sample.

49. The method of claim 48, wherein said amplification employs a polymerase enzyme having 5' to 3' exonuclease activity.

5 50. The method of claim 49, wherein said first and second fluorescent moieties are within no more than 5 nucleotides of each other on said probe.

51. The method of claim 50, wherein said second fluorescent moiety is a quencher.

10 52. The method of claim 48, wherein said *capB* probe comprises a nucleic acid sequence that permits secondary structure formation, wherein said secondary structure formation results in spatial proximity between said first and second fluorescent moiety.

53. The method of claim 52, wherein said second fluorescent moiety is a quencher.

15 54. A method for detecting the presence or absence of *B. anthracis* in a biological sample from an individual or in a non-biological sample, said method comprising:
performing at least one cycling step, wherein a cycling step comprises an
amplifying step and a dye-binding step, wherein said amplifying step comprises contacting said
20 sample with a pair of *capB* primers to produce a *capB* amplification product if a *B. anthracis*
capB nucleic acid molecule is present in said sample, wherein said dye-binding step comprises
contacting said *capB* amplification product with a double-stranded DNA binding dye; and
detecting the presence or absence of binding of said double-stranded DNA
binding dye into said amplification product,

25 wherein the presence of binding is indicative of the presence of *B. anthracis* in said sample, and wherein the absence of binding is indicative of the absence of *B. anthracis* in said sample.

30 55. The method of claim 54, wherein said double-stranded DNA binding dye is selected from the group consisting of SYBRGreenI[®], SYBRGold[®], and ethidium bromide.

56. The method of claim 54, further comprising determining the melting temperature between said *capB* amplification product and said double-stranded DNA binding dye, wherein said melting temperature confirms said presence or absence of said *B. anthracis*.

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